Progress in Oceanography 161 (2018) 87–101

Contents lists available at ScienceDirect

Progress in Oceanography

journal homepage: www.elsevier.com/locate/pocean

Spatio-temporal reproducibility of the microbial food web structure associated with the change in temperature: Long-term observations in the Adriatic Sea

Mladen Šolić[a], Branka Grbec[a], Frano Matić[a], Danijela Šantić[a], Stefanija Šestanović[a,*], Živana Ninčević Gladan[a], Natalia Bojanić[a], Marin Ordulj[b], Slaven Jozić[a], Ana Vrdoljak[a]

[a] Institute of Oceanography and Fisheries, Split, Croatia
[b] University of Split, Department of Marine Studies, Split, Croatia

ARTICLE INFO

Keywords:
Microbial food web
Temperature
Global warming
Reproducibility in time
Long-term data
Self-Organizing Map Analysis
Adriatic Sea

ABSTRACT

Global and atmospheric climate change is altering the thermal conditions in the Adriatic Sea and, consequently, the marine ecosystem. Along the eastern Adriatic coast sea surface temperature (SST) increased by an average of 1.03 °C during the period from 1979 to 2015, while in the recent period, starting from 2008, a strong upward almost linear trend of 0.013 °C/month was noted. Being mainly oligotrophic, the middle Adriatic Sea is characterized by the important role played by the microbial food web in the production and transfer of biomass and energy towards higher trophic levels. It is very important to understand the effect of warming on microbial communities, since small temperature increases in surface seawater can greatly modify the microbial role in the global carbon cycle. In this study, the Self-Organizing Map (SOM) procedure was used to analyse the time series of a number of microbial parameters at two stations with different trophic status in the central Adriatic Sea. The results show that responses of the microbial food web (MFW) structure to temperature changes are reproducible in time. Furthermore, qualitatively similar changes in the structure of the MFW occurred regardless of the trophic status. The rise in temperature was associated with: (1) the increasing importance of microbial heterotrophic activities (increase bacterial growth and bacterial predator abundance, particularly heterotrophic nanoflagellates) and (2) the increasing importance of autotrophic picoplankton (APP) in the MFW.

1. Introduction

Temperature is an extremely influencing factor on microbiological processes such as production (Šolić and Krstulović, 1994; Rivkin et al., 1996; Hoppe et al., 2008), growth rate (White et al., 1991; Shiah and Ducklow, 1994; Šolić et al., 2017) and growth efficiency (Rivkin and Legendre, 2001), as well as grazing on bacteria (Vaqué et al., 1994; Sarmento et al., 2010; Vásquez-Domínguez et al., 2012) and viral lysis (Danovaro et al., 2011; Lara et al., 2013; Maranger et al., 2015; Tsai et al., 2015). However, these processes have shown different sensitivity to temperature increase that ultimately determines how a microbial community will respond to warming (López-Urrutia et al., 2006; Yvon-Durocher et al., 2010). For example, Sarmento et al. (2010) found that warming increases bacterial losses to grazing at lower rates than bacterial production. Furthermore, temperature influences the complex microbial trophic interactions, altering food web structure and ecosystem functioning (Petchey et al., 1999). Since, small changes at the base of the food web could be amplified through trophic chains (Sarmento et al., 2010), investigating the effect of warming on microbial communities is important for better understanding global carbon cycle in seawater. This is particularly important in oligotrophic environments, where microbial food web (MFW) is dominant trophic pathway (Li et al., 1983; Magazzu and Decembrini, 1995; Li, 1998; Li and Harrison, 2001; Grob et al., 2007), such as the eastern coast of the middle Adriatic Sea (Šolić and Krstulović, 1994, 1995; Šolić et al., 2009, 2010, 2015; Šantić et al., 2013).

The semi-enclosed nature of the whole Mediterranean Sea (including the Adriatic Sea), together with its smaller inertia due to the relatively short residence time of its water masses, makes it highly reactive to external forces in particular, global warming. This region, which has been identified as a 'hotspot' for climate change, is therefore expected to experience environmental impacts that are considerably greater than those in many other places around the world (Durrieu de Madron et al., 2011). Recent analysis of the Mediterranean Sea surface temperature showed significant annual warming (from 0.24 °C decade^{-1} west of Strait of Gibraltar to 0.51 °C decade^{-1} over the Black

* Corresponding author at: Institute of Oceanography and Fisheries, POB 500, 21000 Split, Croatia.
E-mail address: sesta@izor.hr (S. Šestanović).

https://doi.org/10.1016/j.pocean.2018.02.003
Received 29 March 2017; Received in revised form 18 January 2018; Accepted 6 February 2018
Available online 07 February 2018
0079-6611/ © 2018 Elsevier Ltd. All rights reserved.

Sea) (Shaltout and Omstedt, 2014). Global climatic models predict that water temperatures will increase by 2–4 °C, on average, over the next few decades (Meehl et al., 2007). There is no doubt that global and atmospheric climate change is altering the thermal conditions in the Adriatic Sea (Matić et al., 2011) and changing the marine ecosystem (Ninčević-Gladan et al., 2009; Grbec et al., 2015).

Experimental manipulation, cross-comparison between marine environments under different temperature regimes (space-for-time substitution analysis) and theoretical predictions are often used as methods for the assessment of the impact of temperature on microbial processes in marine ecosystems. However, long-term observations could be the most direct indicator of changes in the complex trophic interactions within the MFW because of the temperature rise, but long-term data series are relatively rare. In this study, the time series of a number of microbial parameters were analysed at two stations with different trophic status in the central Adriatic Sea. The main objectives of the study were to check the repeatability and reversibility in time of the MFW structure associated with the change in temperature. In particular, we focused on changes in the structure of the MFW in the perspective of global warming. We hypothesized that temperature shifts on decadal scale may cause qualitative changes in the MFW structure, which are reproducible in time and similar on the spatial scale regardless of the difference in the trophic status.

Time series data were analysed using *Self-Organizing Map* (SOM) analysis (Kohonen, 1982, 2001; Kriesel, 2007), which represents an unsupervised clustering method based on a neural network model. The main applications of the SOM are smoothed classification and visualization of complex data usually reduced in size in a two-dimensional space (Kohonen, 1982). SOM has several advantages as compared to other numerical techniques commonly used for classification or gradient analysis because it does not rely on any particular *a priori* assumption on dataset structure; it is not affected by the presence of outliers and can efficiently cope with datasets including a reasonable number of missing data (Giraudel and Lek, 2001). SOM has already been used successfully in atmospheric and ocean sciences (Liu and Weisberg, 2011) for mapping the Adriatic Sea climate (Matić et al., 2017) and classifying the colour of the Adriatic Sea (Kovač et al., 2014), but also for several ecological applications (Lek and Guégan, 1999; Park et al., 2004; Solidoro et al., 2007, 2009; Socal et al., 2008; Bandelj et al., 2008; Choi et al., 2014, 2015; Mazzocchi et al. 2014; Wojtal-Frankiewicz et al., 2015).

2. Material and methods

2.1. Sampling sites

Sampling was conducted at two stations in the central Adriatic Sea, one in the coastal area (Kaštela Bay; KB) and another in the open sea (Stončica; ST) (Fig. 1).

Biological samples were collected from the surface layers during daylight period using 5 L Niskin bottles. At both stations sampling was performed in the same day. All samples employed for cell counts were poured into sterile acid washed glass bottles, fixed immediately (with formaldehyde or glutaraldehyde) and frozen until analysis. The same sampling and counting methods were used throughout the investigation period. SST measurements were performed at Split station (S1) by Marine Meteorological Centre of the Croatian Meteorological and Hydrological Service (Fig. 1).

Kaštela Bay and Stončica stations are included in systematic measurements of different physical, chemical and biological parameters started in the 1950s (Buljan and Zore-Armanda, 1979) which are based on monthly cruises with relatively low number of gaps. Another aspect favourable for the choice of this station is their different trophic status (Šolić et al., 2009), which gave us the opportunity to test whether the effect of temperature on the structure of the microbial food web (MFW) was independent of the trophic status of the studied area.

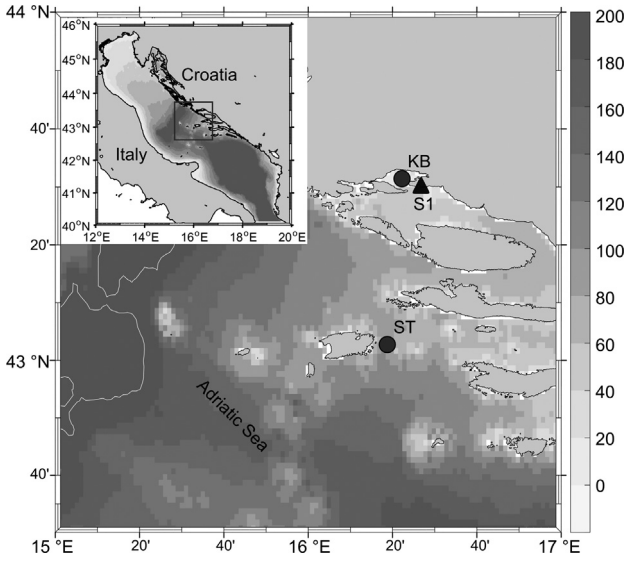

Fig. 1. Studied area with sampling locations. KB (Kaštela Bay) – coastal sea station; ST (Stončica) – open sea station; SST-Split station (S1).

2.1.1. Coastal sea station

The coastal sea station is located in the central part of Kaštela Bay (KB) (43°31′N; 16°22′E), about 3 km from the nearest coast. KB is an enclosed shallow basin with a surface area of 61 km^2 (15 km long and 6 km wide) and an average depth of 23 m that communicates with the adjacent channel through an inlet 1.8 km wide and 40 m deep. The river Jadro, which discharges into the eastern part of the bay, is the most important fresh-water source, with an average annual inflow of 10 m^3 s^{-1} (Orlić et al., 2007). The discharge of several submarine springs and brook Pantan are of lower intensity. Water circulation in the bay is generated mostly by the local wind, which is related to the passage of mid-latitude cyclones over the area (Gačić et al., 1987; Beg Paklar et al., 2002). They are more frequent during the winter period and therefore water circulation and exchange with the open sea are more intense during winter. The average water renewal time is about one month, while under strong wind conditions it can be as short as five days (Zore-Armanda, 1980). During the warm period of the year (July to September) wind forcing is relatively weak and the freshwater inflow low, due to which the renewal time is rather long. Geographical characteristics of the bay, vicinity of land and anthropogenic impact greatly influence the oscillations of chemical and physical parameters (Zore-Armanda, 1980). Precipitation waters also carry considerable quantities of nutrients from land. The eastern part of the bay received large amounts of municipal and industrial effluents, which are significantly reduced after 2005 when the sewage system was completed.

2.1.2. Open sea station

The open sea station, located southeast of Cape Stončica (ST) (43°00′N; 16°20′E) on Vis Island is about 100 m deep and about 50 km offshore. This station is typical of the open middle Adriatic, characterized by smaller oscillations of chemical and physical parameters in comparison to coastal sea (Buljan and Zore-Armanda, 1979).

2.2. Data sets

Time series of microbial parameters listed in Table 1 consist of monthly values throughout the 1997–2015 period, with the exception of the autotrophic picoplankton (APP) groups for which the time series cover the period 2008–2015 (2010–2015 for picoeukaryotes and consequently for total APP biomass). For the period covered by this study, monthly coverage with the measurements of all microbial parameters was larger than 85%, with no gap longer than two consecutive months

Table 1
Time series of microbial data at the coastal (Kaštela Bay) and open sea station (Stončica) collected on the monthly basis (*ciliates were counted only at the coastal sea station). HPP – heterotrophic picoplankton.

Parameter	Units	Period
Variables:		
Heterotrophic bacteria (HB)	cells mL^{-1}	1997–2015
Heterotrophic nanoflagellates (HNF)	cells mL^{-1}	1997–2015
Bacterial production (BP)	µgC L^{-1} day^{-1}	1997–2015
Bacterial specific growth rate (SGR)	day^{-1}	1997–2015
Primary production (PP)	µgC $L^{-1}day^{-1}$	1997–2015
Chlorophyll *a* (CHL)	µgC L^{-1}	1997–2015
Ciliates (CIL)*	ind. L^{-1}	1997–2015
Synechococcus (SYN)	cells mL^{-1}	2008–2015
Prochlorococcus (PROC)	cells mL^{-1}	2008–2015
Picoeukaryotes (PICO)	cells mL^{-1}	2010–2015
Total autotrophic picoplankton (APP)	µgC L^{-1}	2010–2015
Ratios:		
HB/HNF		1997–2015
PP/BP		1997–2015
APP/HPP		2010–2015

and no year with less than 9 samplings. Each measurement always included all studied variables.

2.3. Data collection

2.3.1. Sea surface temperature (SST) measurement

In order to document the long-term SST behaviour in the middle Adriatic Sea, we used monthly average data from daily measurements of SST for the period 1959–2015. SST was measured at 7 h, 14 h, and 21 h (according to local time) at a depth of 30 cm, using a bucket thermometer (Pandžić, 2008). In the middle Adriatic Sea, the long-term SST measurements were performed in several locations including Split station (S1) from 1959 until nowadays without changing observational practices. This unchanged measuring procedure and high frequency of measurement make SST time series suitable for climate research. The continuous monthly averaged SST values obtained from daily measurements at Split station were used in this study, since the long-term variability of SST data is almost uniform over the middle Adriatic Sea (Supić and Orlić, 1992). A nonparametric, locally weighted regression model (LOWESS) was fit to seasonally decomposed data. LOWESS analysis was used to produce a "smooth" set of values from a time series, to clarify the overall non-linear trend of SST data and to observe a strong upward, almost linear trend in the recent period.

2.3.2. Microbial data analysis

2.3.2.1. Flow cytometry analysis. For cell counting undiluted samples were proceed on a Beckman Coulter EPICS XL-MCL cytometer with a flow rate from 1 to 1.2 µL s^{-1}, with analysed volume calculated by acquisition time. According to cytometer performances, we select the flow rate (most usually high flow rate) which allows us to analyze less than 100 particles per second. In order to standardize the fluorescence intensity of the cells, 1.0-µm yellow-green beads were added (Level-III Epics Division of Coulter Corporation Hialeah, Florida).

Abundances of *Synechococcus, Prochlorococcus* and picoeukaryotes were determined according to Marie et al. (1999). 2 mL of preserved samples in 0.5% glutaraldehyde were frozen at -80 °C and stored until analysis (5–10 days). Autotrophic cells were divided into two groups: cyanobacteria (*Synechococcus* and *Prochlorococcus*) and picoeukaryotes, distinguished according to light scattering, red emission of cellular chlorophyll content and orange emission of phycoerythrin-rich cells.

Abundances of Sybr Green-I-stained non-pigmented bacteria were determined according to Marie et al. (1997). Samples for bacteria analysis were preserved in 2% formaldehyde and frozen until analysis (5–10 days). Bacterial abundance was determined in scatter plots of particle side scatter versus Sybr Green I fluorescence related to cellular nucleic acid content to discriminate bacteria from other particles.

Abundances of Sybr Green-I-stained heterotrophic nanoflagellates (HNF) were determined according to Christaki et al. (2011). Abundance was determined in scatter plots of particle side scatter versus Sybr Green I fluorescence related to cellular nucleic acid content to discriminate flagellates from other particles.

2.3.2.2. Picoplankton biomass estimation. Biomasses of studied picoplankton groups were calculated by using the following cell-to-carbon conversion factors: 20 fgC $cell^{-1}$ for heterotrophic bacteria (Lee and Fuhrman, 1987; Kirchman et al., 1993), 36 fgC $cell^{-1}$ for *Prochlorococcus* (Buitenhuis et al., 2012), 255 fgC $cell^{-1}$ for *Synechococcus* (Buitenhuis et al., 2012) and 2590 fgC $cell^{-1}$ for picoeukaryotes (Buitenhuis et al., 2012).

2.3.2.3. Bacterial production. Bacterial cell production was estimated by measuring the incorporation of ^{3}H-thymidine into bacterial DNA (Fuhrman and Azam, 1982). Methyl-^{3}H-thymidine was added to 10 mL samples at a final concentration of 10 nmol (specific activity: 86 Ci $mmol^{-1}$). Triplicate samples and a formaldehyde-killed adsorption control (final concentration: 0.5%) were incubated for 1 h. The incubations were stopped with formaldehyde (final concentration: 0.5%). The thymidine samples were extracted with ice-cold trichloroacetic acid (TCA), according to Fuhrman and Azam (1982). The TCA-insoluble fraction was collected by filtering the samples through 0.2 µm pore size polycarbonate filters. From the estimates of bacterial production (BP) and bacterial biomass (BB), bacterial specific growth rate (SGR, day^{-1}) was computed as: SGR = ln (1 + BP/BB).

2.3.2.4. Chlorophyll a. Chlorophyll *a* concentrations were determined fluorometrically from 90% acetone extracts (Strickland and Parsons, 1972). Seawater subsamples (500 mL) were filtered through Whatman GF/F glass-fibre filters and the filters were frozen until the analysis carried out within one month of sampling. Filters were homogenised and extracted in 90% acetone for 2 h in the dark at room temperature and analysed using a Turner TD-700 Laboratory Fluorometer.

2.3.2.5. Primary production. Primary production was measured by a ^{14}C tracer technique according to Steemann-Nielsen (1952). Seawater samples (100 mL) were collected in light and dark bottles from different depths and ampoules of $^{14}CO_2$ were added in the form of $NaH^{14}CO_3$. Samples were returned to the depth where they were collected and incubated in situ for 6 h. One light and one dark bottle were incubated at each depth. After incubation, samples were filtered through membrane filters with 0.2 µm pore size. The amount of $^{14}CO_2$ assimilated was determined by measuring β radiation from the phytoplankton retained on the filter. All of the radioactivity measurements of our samples were performed at C-14 Centralen in Denmark.

2.3.2.6. Ciliates. Sample volumes of 5 L were sedimented (Utermöhl, 1958) for 48 h in plastic containers and decanted down to a volume of 2 L. This volume was poured into a cylinder and sedimented for 48 h. The excess volume was then reduced to 200 mL. Prior to microscopic analysis, the volume was further reduced to 20 mL. Decanting was carried out using a vacuum pump and a slightly curved pipette that removed water from the surface. Counting and species identification were carried out in a glass chamber (76 × 47 × 6 mm) using inverted microscopes (Olympus IMT-2 and CK40) at 100× and 400× magnification. The entire bottom of the sedimentation chamber was analysed and abundance was expressed as number of cells per litre. The ciliates were preserved in 2.5% formaldehyde-seawater solution, previously buffered with $CaCO_3$, since Lugol's solution stains detritus that can be abundant in the eutrophicated area and would thus reduce visibility (Fonda-Umani and Beran, 2003). However, formaldehyde

causes cell loss of nonloricate ciliates (Leakey et al., 1994) and therefore their abundances may be somewhat underestimated.

2.3.2.7. Viruses. The abundance of marine viruses was determined as described in Noble and Fuhrman (1998), with slight adjustments. Collected samples were preserved in formaldehyde (2%, final concentration) and frozen at −80 °C until analysis, which was performed in the laboratory immediately at the end of the cruise. Preserved samples (2 mL) were filtered onto 0.02-μm pore-size filters (Anodisc; diameter: 25 mm; Al_2O_3, Whatman) and stained with Sybr Green I (stock solution diluted 1:300). Filters were incubated in the dark for 20 min and mounted on glass slides with a drop of 50% phosphate buffer (6.7 mM, pH 7.8) and 50% glycerol, containing 0.5% ascorbic acid. Slides were stored at temperature of −20 °C until analysis. Viral counts were obtained by epifluorescence microscopy (Olympus BX 51, 1250× magnification, equipped with blue excitation filter) and were expressed as the number of virus-like particles (VLP).

2.4. Statistical analysis

2.4.1. Self-Organizing Maps method (SOM)

SOM is a neural network technique (Kohonen, 1982), trained using unsupervised competitive learning, that combines k-means clustering and data smoothing. The method reduces dimensionality of the input data space on a prescribed number of neurons, usually located on a two-dimensional grid.

During SOM learning process, iteration algorithm tries to find the minimum Euclidean distance between the node's weight vectors (neurons) and data vectors, preserving topological characteristics. At the end of the iterative procedure the weight vector with minimum distance from data vector is identified as the best matching unit (BMU) of the input vector. SOM aims at maximizing the similarity between the patterns it represents by neurons and the patterns actually present in the data (Kohonen, 2001, 2014).

One of SOM results is the U-matrix that shows the Euclidean distances between weight vectors on the map. SOM gives two measures of mapping quality: average quantization error (QE) and topographic error (TE), describing average distance between data vector and BMU and percentage of the data vectors for which the first BMU and the second BMU are not neighbouring units (Liu et al., 2006).

For application of SOM, it is necessary to select the number of neurons of the training algorithm (training length, neighbourhood size and learning rate) and number of neurons i.e. size of SOM array. The number of neurons is the crucial parameter affecting modelled variance of the data. However, there is no generally accepted method how to determine the number of neurons. In this paper, we applied a two-step procedure (Vesanto and Alhoniemi, 2000) and further clustered the SOM vectors.

In this study, the input data for SOM model is a collection of samples, with each sample containing monthly values of MFW parameters. Each microbial time series was logarithmically transformed and normalized using the respective seasonal mean and standard deviation, which is a prerequisite for avoiding variation and skewness, and equal treatment of different parameters using SOM analysis (Kohonen, 2001).

SOM analysis was used in the following combination: the hexagonal neural lattice of “sheet” shape, the linear SOM initialization, the “ep” neighbourhood function and the batch-training algorithm. The number of iterations was set to “long”. This option was chosen following recommendations from previous SOM studies (e.g. Liu et al., 2006, Solidoro et al., 2007). The SOM array was set to 5 × 4 (20 clusters).

The analysis was performed using the original SOM Toolbox downloaded from http://www.cis.hut.fi/projects/somtoolbox.

2.4.2. Cluster analysis

The hierarchical cluster analysis was used to investigate relationships among BMU-s based on the group average clustering from Bray-Curtis similarity matrix of standardized and log transformed biological data matrix. Application of “Similarity profile” (SIMPROOF) permutation test allowed the identification of the significant internal structure within the constructed clusters, and this option was used to test the significance of the output dendrogram (at 1000 permutations). The test results are displayed by colour convention on the dendrogram plot (Fig. A1c and A3c in Appendix A) and by the test statistics (π value), with a significance level set at $p = 0.01$. These analyses were performed using the statistical package PRIMER 6 (version 6.1.13) (Clarke and Gorley, 2006).

3. Results and discussion

3.1. Sea surface temperature variability

An upward SST trend is observed along the eastern Adriatic coast after 1979. SST increased by an average of 1.03 °C during the period from 1979 to 2015 (Fig. 2). This is in accordance with the sea surface warming trend observed in other Mediterranean areas (Skliris et al., 2011; Shaltout and Omstedt, 2014; Schroeder et al., 2016). A positive trend was more pronounced in the last few decades, which prompted us to study the ecosystem responses further. For the period from1997 to 2015, a non-linear upward trend was obtained, while in the recent period, starting from 2008, a strong upward almost linear trend of 0.013 °C per month was noted (Fig. 2). During this period, SST increased by 1.25 °C.

3.2. Reproducibility of the MFW structure over the 1997–2015 period

After SOM analyses were performed on coastal (Kaštela Bay) and open sea (Stončica) station data (see Appendix A), the best matching units (BMU) vectors were clustered using Bray-Curtis similarity method (Fig. A1) into 4 significantly different ($p = 0.001$) clusters (CL-A, CL-B, CL-C and Cl-D) representing different patterns of the MFW structure. The mean values of MFW variables for each cluster are shown in Table 2 (the results of the Tukey's HSD post hoc test are listed in Suplementary material).

CL-A describes MFW structure characterized by extreme values of heterotrophic components of the MFW, which dominated over the autotrophic components. The main features of the MFW that belong to CL-A included high values for bacterial growth (BP and SGR) and HNF abundance at both stations, and low values for PP/BP ratio at both stations and for PP at the open sea station.

CL-B describes a more stable MFW status with a much smaller number of variables that showed extreme values. High value was established only for HB/HNF ratio at the coastal site, and low values for PP at both sites and for HNF at the coastal site.

MFW represented by CL-C is mostly opposite to that represented by CL-A, showing low values for heterotrophic components of the MFW (BP, SGR, HNF). On the other hand, high values were established for PP/BP and HB/HNF at both sites, as well as for PP at the coastal site and for HB at the open sea site.

CL-D describes MFW structure characterized by high values of PP and HNF abundance. On the other hand, low values were established for HB and HB/HNF ratio.

3.2.1. Successions of clusters in time and relationship with temperature

Successions of clusters in time, together with temperature changes, are presented in Fig. 3.

Although temperature in the Adriatic Sea has been following an upward trend since the 1970 s (Fig. 2), the period from 1997 to 2015 was characterized by alternating changes, revealing positive and negative temperature anomalies. Time series of smoothed (LOWESS algorithm) sea surface temperature pointed to three periods characterized by different average temperature: (1) 1998–2004 period was characterized by higher average temperature (17.77 °C); (2) after 2004

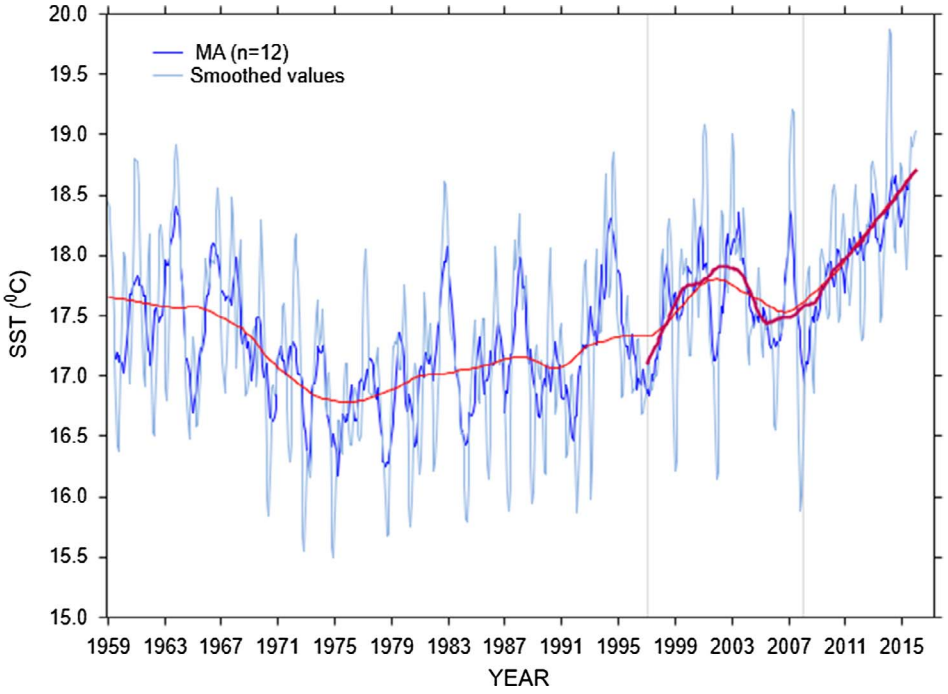

Fig. 2. Seasonal decompositions of mean monthly sea surface temperature for the middle Adriatic Sea obtained from moving averages procedure. Non-linear trend for the period 1959–2015 (thin red line) and for the period 1997–2015 (bold red line) are presented as LOWESS spline. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

temperature decreased and lower average temperature (17.48 °C) lasted until 2009; (3) after 2009 temperature started to increase, reaching particularly high average values after 2013 (18.37 °C). These three temperature averages were calculated from monthly means with previously removed seasonal component, and they were statistically significantly different (ANOVA; $p < 0.01$).

At the coastal site (Kaštela Bay), the timeline of clusters started with the dominance of CL-A (72% of all clusters in period from 1997 to 2001), which coincided with the period of higher average temperature. After 2001, the dominant MFW structure mostly alternated between CL-B and CL-C (86% of all clusters in period from 2001 to 2011). This condition lasted until 2012, coinciding with the period of lower average temperature. After 2012, the MFW structure became dominantly represented by CL-D (64% of all clusters in period from 2012 to 2015), which coincided with significant increase in temperature. A similar time sequence of clusters was found for the open sea site (Stončica). In comparison to the coastal site, at the open sea site, during the period of lower average temperature, the MFW structure was more frequently represented by CL-C. Further, CL-D became dominant two years earlier (after 2010) at the open sea site. The alternation between the periods of higher and lower average temperature throughout the studied period corresponded to the change of MFW structure (represented by four clusters), and this process was similar for both studied areas despite the differences in their trophic status. This is supported by the study in the Adriatic Sea which reported that availability of phosphorus affected the BP, but did not change the nature of the relationship between BP and temperature (Šolić et al., 2017).

Table 2
Microbial food web parameter means per cluster as a result of group average clustering from Bray-Curtis similarity matrix on BMU from SOM analysis. The significant differences between clusters were based on One-way ANOVA analysis (F – false value; *P* – probability value). HB – heterotrophic bacteria; BP – bacterial production; SGR – bacterial specific growth rate; HNF – heterotrophic nanoflagellates; PP – primary production; CHL – chlorophyll *a*.

Variables	CL-A	CL-B	CL-C	CL-D	F	*p*
Kaštela Bay						
Log HB (cells mL^{-1})	5.95	5.92	5.86	5.77	14.15	0.000091
Log HNF (cells mL^{-1})	3.35	2.79	3.05	3.45	14.01	0.000097
BP (μgC $L^{-1}day^{-1}$)	2.76	1.47	1.14	1.53	38.85	0.000000
SGR (day^{-1})	0.55	0.20	0.12	0.28	48.05	0.000000
PP (μgC $L^{-1}day^{-1}$)	35.84	23.21	41.49	34.44	8.05	0.001705
CHL (μgC L^{-1})	0.66	0.60	0.74	0.71	1.37	n.s.
Log (HB/HNF)	2.62	3.12	2.80	2.31	13.96	0.000099
PP/BP	5.11	15.42	40.31	15.69	38.30	0.000000
Stončica						
Log HB (cells mL^{-1})	5.60	5.57	5.72	5.54	7.13	0.002951
Log HNF (cells mL^{-1})	2.90	2.82	2.32	2.87	12.90	0.000154
BP (μgC $L^{-1}day^{-1}$)	2.23	1.29	0.99	0.95	64.00	0.000000
SGR (day^{-1})	0.78	0.34	0.13	0.16	58.25	0.000000
PP (μgC $L^{-1}day^{-1}$)	3.50	4.37	4.50	6.97	13.61	0.000114
CHL (μgC L^{-1})	0.10	0.10	0.13	0.16	2.48	n.s.
Log (HB/HNF)	2.70	2.75	3.39	2.67	11.06	0.000353
PP/BP	0.55	2.41	4.80	6.81	51.10	0.000000

n.s. – not significant ($p > 0.05$).

The comparison of the warm period 1997–2004 to the even warmer period after 2013 showed some differences in the MFW structure. The period 1997–2004 was characterized by very high values of bacterial growth (BP and SGR) that had never been reached since. High bacterial growth during this period could be explained by larger amounts of nutrients. Until 2005, Kaštela Bay received high quantities of organic matter and nutrients due to the discharge of untreated sewage waters. In November 2004, the sewage system was completed. The switch from higher to lower trophic status was accompanied by decreases in HB, BP and CHL, and increase in HNF abundance and SGR (Šolić et al., 2010). On the other hand, the study at the open sea station (Stončica) showed that HB and BP were controlled by different water mass dynamics during 1997–2001 compared to the 2002–2006 period, which resulted in a change of available nutrients (Šolić et al., 2008). Both studies suggested strong bottom-up control of bacteria.

During the warm period after 2013, bacterial growth (BP and SGR) started to increase, but it did not reach values as high as they were in the period 1997–2004. Non-linear responses of bacterial growth to temperature that have shown greater positive effect at lower temperatures, followed by reaching a plateau or even a decrease at higher temperatures, were reported for different marine and freshwater environments (Ratkowsky et al., 1982; Felip et al., 1996; Pomeroy and Wiebe, 2001; Apple et al., 2006; Vásquez-Domínguez et al., 2012). Our previous study in the Adriatic Sea also showed non-linear relationship between bacterial growth and temperature, but linear increase of HNF

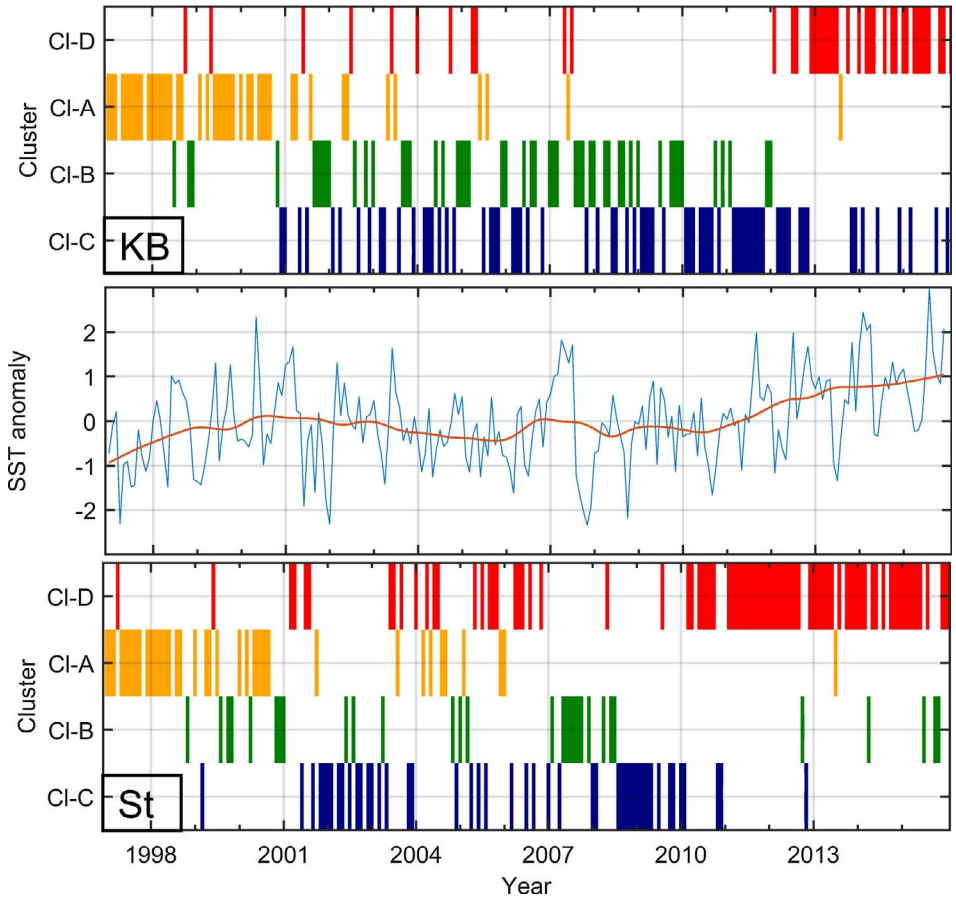

Fig. 3. Time series of normalized monthly means (anomalies) of SST (blue line), smoothed SST using LOWESS algorithm (red line) (middle plot). Time series of clusters (CL-A, CL-B, CL-C, CL-D) at Kaštela Bay (KB) and Stončica (St). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

grazing on bacteria and HNF abundance (Šolić et al., 2017). This is in accordance with the MFW structure represented by CL-D, which showed high HNF abundance suggesting domination of top-down control of bacteria.

Periods of higher and lower average temperature corresponded to the dominance of a particular cluster, which represented a particular type of MFW structure. However, during these periods a pronounced temperature oscillation on a relative short time scale occurred occasionally. Our results show that the MFW promptly responded to these short-term changes, repeatedly changing structural features (Fig. 3).

The period 2004–2009, which was characterized by the lower average temperature (Fig. 3), is a good example. During this period, the dominant structural state of the MFW was represented by CL-B and/or CL-C. However, almost every peak of temperature during this period was accompanied by a switch of the MFW structure from the state represented by CL-B and/or CL-C to the state represented by CL-D. CL-D became dominant later (after 2012), when the temperature increased and stabilized at a higher value. Similar responses of the MFW structure were observed during marked decreases in temperature, in 1999 and 2002, which occurred during the period of higher average temperature. In fact, strong bora events produced an unusually cold January and February in 1999, and an extremely cold January in 2002. In both cases, atmospheric conditions over the Adriatic significantly reduced the heat content of the sea surface layer. Average heat loss during the first three months of year 1999 was close to 300 Wm^{-2} (Manca et al., 2002). In January 2002, sea surface temperature was significantly below the climatic average. These events were followed by a switch of the MFW structure from CL-A to CL-B or CL-C (Fig. 3).

3.3. Reorganization of the MFW structure associated with the significant rise in temperature during the period 2008-2015

A more detailed analysis of changes in the structure of the MFW that can be expected in terms of global warming, has been performed for the period 2008–2015, because this period is characterized by a continuous and significant rise in surface seawater temperature (SST) (Fig. 2) and a larger number of microbial parameters were available. During this period, annual mean SST increased by 1.25 °C or 0.90% per year (significant according to Mann-Kendall test; $p < 0.01$). Furthermore, statistically significant upward or downward trends for the number of microbial parameters were established, showing a statistically significant (positive or negative) correlation with temperature (Table 3). HB showed particularly strong negative correlation, while HNF and APP showed a strong positive correlation with temperature. Therefore, HNF/HB ratio increased with temperature suggesting strong top-down control of bacteria, which is in accordance with the previous study in the Adriatic Sea (Šolić et al., 2017).

After SOM analyses were performed on coastal (Kaštela Bay) and open sea (Stončica) station data (see Appendix A), the best matching units (BMU) vectors were clustered using Bray-Curtis similarity method (Fig. A3) into statistically significantly different ($p = 0.001$) clusters (CL-1, CL-2 and CL-3) and sub-clusters (CL-1A, CL-1B, CL-2A, CL-2B, CL-3A and CL-3B) for both SOM models (Fig. A3). The mean values of MFW variables for each cluster are shown in Table 4 (the results of the Tukey's HSD post hoc test are listed in Suplementary material).

CL-1 is characterized by low values of bacterial growth (BP and SGR), HNF abundance, abundance of APP groups (SYN, PICO, PROC and total APP biomass), autotrophic to heterotrophic picoplankton ratio (APP/HPP) and high value of PP/BP ratio. The most important differences between sub-clusters CL-1A and CL-1B are considerably higher

Table 3
Regression statistics of biological parameters and SST with time in months (b – slope of regression; R^2 – coefficient of determination; *p* – significance level; change – average annual percentage change in the values of the studied parameters) and Pearson's correlation coefficients between biological parameters and SST (r), for 2008–2015 period. (SST – sea surface temperature; HB – heterotrophic bacteria; HNF – heterotrophic nanoflagellates; BP – bacterial production; SGR – bacterial specific growth rate; PP - primary production; CHL – chlorophyll *a*; SYN – *Synechococcus*; PROC – *Prochlorococcus*; PICO – picoeukaryotes; APP – total autotrophic picoplankton biomass; HPP – heterotrophic picoplankton biomass; CIL – ciliates).

Time series	Kaštela Bay					Stončica				
	Regression statistics				Corr. with SST	Regression statistics				Corr. with SST
	b	R^2	p	Change	r**	b	R^2	p	Change	r**
Parameters:										
SST* (°C)	0.0151	0.77	< 0.0001	0.9	–	0.0151	0.77	< 0.0001	0.9	–
Log HB (cells mL^{-1})	−0.0032	0.775	< 0.0001	−6.39	−0.801[1]	−0.0046	0.754	< 0.0001	−7.96	−0.725[1]
Log HNF (cells mL^{-1})	0.0115	0.848	< 0.0001	129.75	0.814[1]	0.0099	0.786	< 0.0001	89.41	0.564[1]
BP (μgC $L^{-1}day^{-1}$)	0.0078	0.084	0.0027	6.67	0.452[1]	0.0083	0.193	< 0.0001	14.68	0.426[1]
SGR (day^{-1})	0.0019	0.425	< 0.0001	33.71	0.534[1]	0.0026	0.6	< 0.0001	89.18	0.661[1]
PP (μgC $L^{-1}day^{-1}$)	–	0.008	n.s.	–	n.s.	0.0302	0.135	0.0002	5.09	0.424[1]
CHL (μgC L^{-1})	–	0.009	n.s.	–	n.s.	–	0.009	n.s.	–	n.s.
Log SYN (cells mL^{-1})	0.0174	0.875	< 0.0001	394.54	0.852[1]	0.0114	0.407	< 0.0001	124.56	0.423[1]
Log PROC (cells mL^{-1})	0.0099	0.462	< 0.0001	73.61	0.741[1]	–	0.049	n.s.	–	n.s.
Log PICO (cells mL^{-1})	0.0142	0.838	< 0.0001	120.24	0.699[1]	0.0141	0.565	< 0.0001	118.32	0.615[1]
APP (μgC L^{-1})	1.69	0.892	< 0.0001	143.39	0.705[1]	0.3316	0.694	< 0.0001	31.17	0.636[1]
Log CIL (cells L^{-1})	0.0012	0.353	< 0.0001	3.75	n.s.	–	–	–	–	–
Ratios:										
Log (HB/HNF)	−0.0148	0.866	< 0.0001	−12.57	−0.810[1]	−0.015	0.864	< 0.0001	− 12,60	−0.716[1]
PP/BP	−0.7287	0.724	< 0.0001	−11.52	−0.791[1]	−0.0862	0.246	< 0.0001	−7.56	−0.416[1]
APP/HPP	0.2018	0.894	< 0.0001	387.43	0.653[1]	0.0593	0.735	< 0.0001	69.99	0.553[1]

n.s. – not significant.

* Data from marine meteorological centre of the croatian meteorological and hydrological service.

** Pearson's correlation coefficients and significant level were calculated following Manly, 1997 and Groppe et al., 2011 with 5000 permutations and desired family-wise alpha level 0.01.

[1] $p < 0.01$.

values of bacterial growth (BP and SGR) and APP/HPP ratio in the CL-1A at both stations. Further, PP was higher in the CL-1A at Stončica and in the CL-1B in Kaštela Bay.

CL-2 shows no specific pattern of MFW variables. We suggest that this is a transitional category, because most variables were intermediate in both numerical scale and topological position on the map. However, transition from sub-cluster CL-2A to sub-cluster CL-2B was accompanied with qualitative changes in the most of the variables in the sense that positive anomalies switched to negative, and *vice versa*.

MFW represented by CL-3 is mostly opposite to that represented by CL-1. The main features of the MFW that belong to CL-3 included high values of HNF, SYN, PROC, PICO, APP and APP/HPP and low values of HB, HB/HNF and PP/BP. The most important differences between sub-clusters CL-3A and CL-3B are considerably higher values of bacterial growth (BP and SGR) in the CL-3A at Stončica and in the CL-3B in Kaštela Bay. Further, APP biomass was considerably higher in the CL-3B.

In general, the increase in temperature during the 2009–2015 period was accompanied by a succession of clusters from CL-1 over the CL-2 to CL-3, showing gradual reorganization in the structure of the MFW (Fig. 4).

One set of mutually connected changes, associated with temperature increase, entailed an increase of bacterial growth (BP and SGR) and abundance of HNF as most important bacterial predators. HNF increased 130% per year at the coastal sea station and 89% per year at the open sea station during the investigation period (Table 3). Contrary, bacterial abundance (HB) decreased with temperature. Consequently, throughout this period the HB/HNF ratio decreased by almost one order of magnitude.

The fact that during warming conditions HB did not increase with increasing BP, and that the increase in bacteriovorous protozoa (HNF) occurred at the same time, suggests that rising temperatures have led to strong top-down control of bacteria. Billen et al. (1990) showed that all bacterial production can be converted into bacterial biomass when grazing pressure on bacteria is low. Alternatively, if grazing on bacteria is very high, bacterial biomass does not increase with increasing bacterial production. As a result of increasing bacterial growth and the likely increase

Table 4
Microbial food web parameter means per cluster as a result of group average clustering from Bray-Curtis similarity matrix on BMU from SOM analysis. The significant differences between clusters were based on One-way ANOVA analysis (F – false value; *P* – probability value). HB – heterotrophic bacteria; BP – bacterial production; SGR – bacterial specific growth rate; HNF – heterotrophic nanoflagellates; PP – primary production; CHL – chlorophyll *a*; SYN – *Synechococcus*; PROC – *Prochlorococcus*; PICO – picoeukaryotes; APP – total autotrophic picoplankton biomass; HPP – heterotrophic picoplankton biomass; CIL – ciliates.

Variables	CL-1	CL-2	CL-3	F	*p*
Kaštela Bay					
Log HB (cells mL^{-1})	5.84	5.77	5.70	42.41	0.000251
Log HNF (cells mL^{-1})	2.85	3.12	3.42	35.95	0.000015
BP (μgC $L^{-1}day^{-1}$)	1.06	1.17	1.27	13.31	0.000025
SGR (day^{-1})	0.08	0.12	0.16	22.38	0.000001
PP (μgC $L^{-1}day^{-1}$)	31.11	30.47	33.90	11.54	n.s.
CHL (μgC L^{-1})	0.56	0.56	0.71	12.35	n.s.
Log SYN (cells mL^{-1})	4.23	4.60	4.90	20.67	0.000007
Log PROC (cells mL^{-1})	4.42	4.62	4.84	16.38	0.000183
Log PICO (cells mL^{-1})	3.20	3.47	3.72	26.58	0.000001
APP (μgC L^{-1})	26.42	50.11	69.44	19.46	0.000000
Log CIL (cells L^{-1})	2.26	2.25	2.27	1.63	n.s.
Log (HB/HNF)	2.97	2.63	2.27	40.73	0.000014
PP/BP	46.52	32.90	22.00	14.67	0.000020
APP/HPP	1.95	4.57	7.13	25.91	0.000000
Stončica					
Log HB (cells mL^{-1})	5.52	5.71	5.49	28.32	0.005957
Log HNF (cells mL^{-1})	2.86	2.51	3.05	24.14	0.001218
BP (μgC $L^{-1}day^{-1}$)	0.86	0.87	1.11	25.67	0.000006
SGR (day^{-1})	0.10	0.07	0.19	40.21	0.000005
PP (μgC $L^{-1}day^{-1}$)	9.52	5.82	6.96	11.75	0.001204
CHL (μgC L^{-1})	0.13	0.15	0.14	2.73	n.s.
Log SYN (cells mL^{-1})	3.02	3.25	3.74	37.92	0.000066
Log PROC (cells mL^{-1})	3.12	3.53	3.40	13.56	0.032517
Log PICO (cells mL^{-1})	2.94	3.19	3.48	31.04	0.000016
APP (μgC L^{-1})	2.96	6.17	12.14	30.36	0.000040
Log (HB/HNF)	2.65	3.20	2.46	22.86	0.001990

(*continued on next page*)

Table 4 (*continued*)

Variables	CL-1	CL-2	CL-3	F	*p*
PP/BP	12.29	8.33	4.71	33.30	0.000221
APP/HPP	0.45	0.64	2.04	59.77	0.000000

n.s. – not significant ($p > 0.05$).

of grazing on bacteria (GB), bacterial biomass turnover significantly accelerated with increasing temperature. During the 2008–2015 period, average bacterial biomass turnover decreased from 16.5 to 4.6 days (regression statistics with temperature: $b = -8.88$; $F_{1,83} = 122.64$; $r = -0.772$; $p < 0.001$), and from 20.4 to 2.3 days (regression statistics with temperature: $b = -17.34$; $F_{1,83} = 172.87$; $r = -0.822$; $p < 0.001$) at the coastal and open sea station, respectively.

These results are in accordance with the *metabolic theory of ecology* (MTE) (Brown et al., 2004), which takes the individual metabolic rate as a fundamental variable regulating the flux of energy and matter through different levels of the food web. According to MTE one would expect both BP and GB to increase with temperature, but with GB increasing at a higher rate than BP. Results of the previous studies in the Adriatic Sea confirmed a strong increase of GB with increasing temperature (Šolić and Krstulović, 1994, 1995; Šolić et al., 2017). Moreover, with increasing temperature GB would be tightly coupled to BP, which means that a larger proportion of BP would be taken by grazers. Therefore, MTE predicts a decrease of HB with increasing temperature (West et al., 1997; Bernacchi et al., 2001; Enquist et al., 2003; Brown et al., 2004; Allen et al., 2005; López-Urrutia et al., 2006). A decrease of HB with increasing temperature was reported in recent years for the Mediterranean Sea (Sarmento et al., 2010). Gallina et al. (2011) also reported a negative correlation between temperature and HB in the Adriatic Sea, but this relationship varied seasonally. The observed changes within heterotrophic microbial components suggest that temperature rises could favour the consumption and cycling of organic matter in the surface layer through prokaryotic heterotrophic metabolism, and could increase the proportion of BP transferred to the metazoan food web (Šolić et al., 2017).

Increase of viral lysis with temperature has been also reported (Proctor et al., 1993; Danovaro et al., 2011). In our study, VLP showed no correlation with temperature at the coastal station, whereas at the open sea station VLP decreased with increasing temperature ($b = 0.085$; $F_{1,19} = 45.72$; $r = 0.912$; $p < 0.0001$). This could be explained by tight coupling of viruses with HB, which also decreased with temperature, with a more expressed rate of decrease at the open sea station compared to the coastal sea station (regression slopes were $b = -0.381$ and $b = -0.201$, respectively).

Autotrophic components of MFW also changed with temperature increase. A strong increasing trend of total APP biomass was established at both stations, showing significant positive relationship with temperature (Table 3). There is general agreement that a rise in temperature increases the domination of pico-phytoplankton in biomass and production (Strecker et al., 2004; Daufresne et al., 2009; O'Connor et al., 2009; Morán et al., 2010). Daufresne et al. (2009) reported that an increase in temperature favours smaller organisms in aquatic systems for a given level of resources, leading to a higher proportion of pico-phytoplankton among the autotrophs (Morán et al., 2010) and increasing heterotrophic bacterial activity (Iriberri et al., 1985; White et al., 1991).

The contribution of SYN, PROC and PICO biomasses to total APP biomass differed between the two stations. The biomass of cyanobacteria (SYN + PROC) dominated over that of picoeukaryotes at the coastal sea station, while the biomass of picoeukaryotes was dominant at the open sea station. During the studied period, the contribution of SYN biomass to total APP biomass increased at both stations (about 10% in KB and about 20% at ST), mostly to the detriment of PICO (Fig. 5). An increasing trend of APP

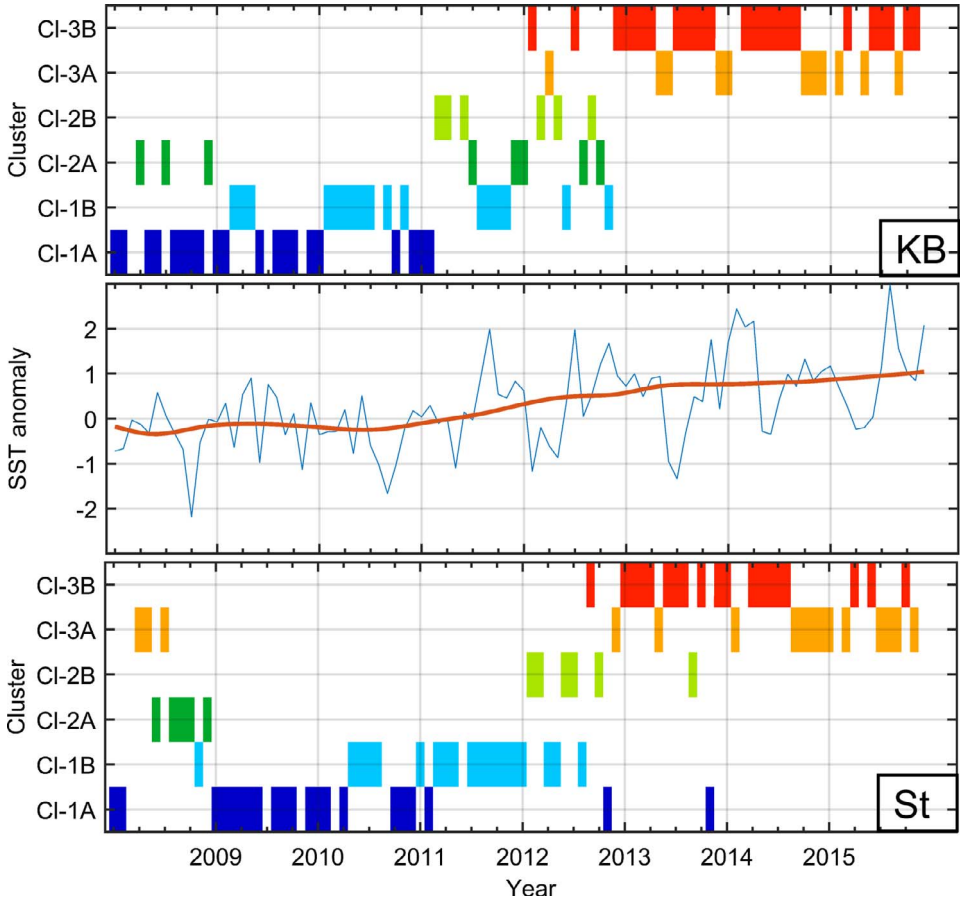

Fig. 4. Time series of normalized monthly means (anomalies) of SST (blue line), smoothed SST using LOWESS algorithm (red line) (middle plot). Time series of sub-clusters (CL-1A, CL-1B, CL-2A, CL-2B, CL-3A, CL-3B) at Kaštela Bay (KB) and Stončica (St). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

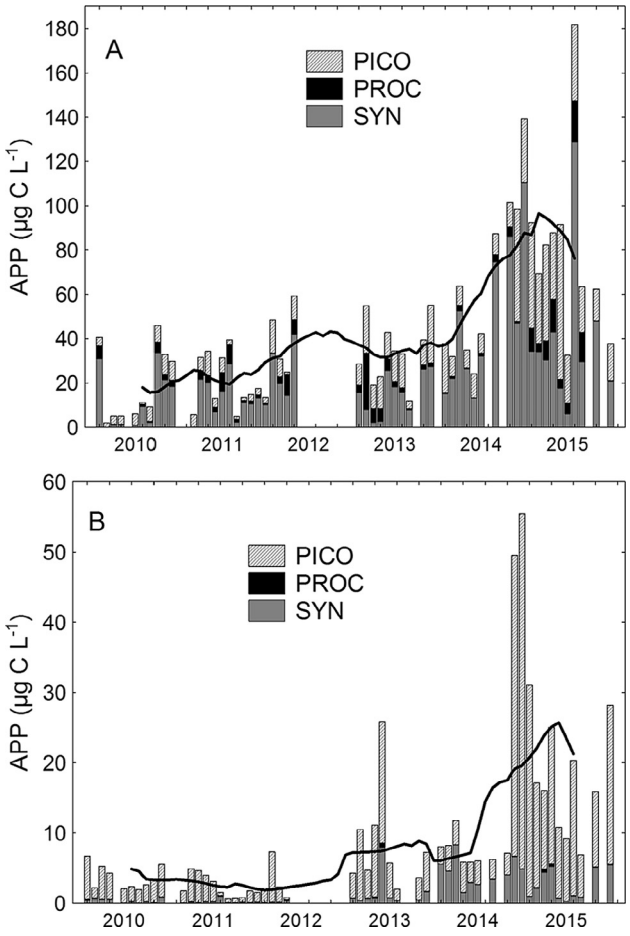

Fig. 5. Biomass distribution of *Synechococcus* (SYN), *Prochlorococcus* (PROC) and picoeukaryotes (PICO) throughout the 2010–2015 period at coastal (A) and open sea (B) station. Lines represented time series of the total APP biomass detrended for seasonal patterns (12-months moving averages).

biomass, and decreasing trend of bacterial biomass resulted in a strong increasing trend of the ratio between autotrophic and heterotrophic picoplankton (APP/HPP) at both stations.

PP and CHL showed no correlation with temperature, with exception of PP at the open sea station where statistically significant but not very strong correlation with temperature was established (Table 3). Moreover, CHL was the only parameter which did not show statistically significant difference among the clusters in both investigated periods. A possible explanation for this is that the CHL was not measured separately for different size fractions. Therefore, the simultaneous increase of pico-phytoplankton biomass and decrease of large phytoplankton biomass with temperature rise could result in a relatively stable overall concentration of CHL.

At both stations the ratio between primary production and bacterial production (PP/BP) decreased with temperature. Experimental studies of the warming effect on the microbial community in Arctic systems showed that heterotrophic and phototrophic microbial communities responded differentially to warming conditions, with significant increase in the activities and biomass of heterotrophic microorganisms and decrease in phytoplankton biomass (Lara et al., 2013). Satellite chlorophyll records indicate that global annual ocean primary production has decreased by 6% since the early 1980s (Gregg and Conkright, 2002). Most of the decrease occurred at high latitudes (Sarmiento et al., 1998; Behrenfeld et al., 2006).

4. Conclusion

This study is based on the long-term observations of the relationship between temperature and the MFW structure in the Adriatic Sea. The results of Self-Organizing Map (SOM) analysis showed that responses of the MFW structure to temperature changes were reproducible in time. Furthermore, qualitatively similar changes in the structure of the MFW occurred regardless of trophic status. The rise in temperature was associated with: (1) increasing importance of microbial heterotrophic activities, and (2) increasing importance of APP in the MFW. The temperature increase was accompanied with an increase in bacterial growth, bacterial biomass turnover, bacterial predator abundances (particularly HNF). Simultaneously, autotrophic picoplankton (APP) biomass (including *Synechococcus, Prochlorococcus* and picoeukaryotes) significantly increased with temperature, suggesting that APP have a great potential as additional carbon biomass that can be channelled to higher trophic levels under the warming conditions.

Acknowledgements

This research was supported by the Croatian Science Foundation as a part of research projects: IP-2014-09-4143 “Marine microbial food web processes in global warming perspective” (MICROGLOB) and IP-2014-09-3606 “Marine plankton as a tool for assessment of climate and anthropogenic influence on the marine ecosystem” (MARIPLAN). SST data was obtained thanks to ViLab project (http://www.izor.hr/web/guest/virtual-laboratory).

Appendix A

A.1. Ordination of sampling terms with the Self-Organizing Map (SOM) model

Four SOM models were performed, one for the coastal (KB) and one for the open sea (ST) for two studies periods (1997–2015 and 2008–2015). Characteristics of SOM models are listed in Table A1. SOM models include samples with 8 and 14 microbial food web (MFW) variables for 1997–2015 and 2008–2015 periods, respectively.

Table A1
Characteristic and errors of SOM models all set as follows: The 5 × 4 SOM array, the hexagonal neural lattice of “sheet” shape, the linear SOM initialization, the “ep” neighbourhood function, and the batch training algorithm.

Period	Station	Number of time samples	MFW variables number	Quantization error	Topographic error
1997–2015	KB	193	8	1.51	2.4%
1997–2015	ST	193	8	1.47	4.9%
2008–2015	KB	84	14	2.32	0.5%
2008–2015	ST	84	14	1.92	1.2%

A.1.1. SOM models for 1997-2015 period

After training both SOM models for 1997–2015 period resulting with U-matrix (Fig. A1a) and trained SOM component map (Fig. A2), the BMU vectors were clustered using Bray-Curtis similarity method (Fig. A1c) into 4 significantly different (p = 0.001) clusters (CL-A, CL-B, CL-C and Cl-D) for both SOM models (Fig. A1b).

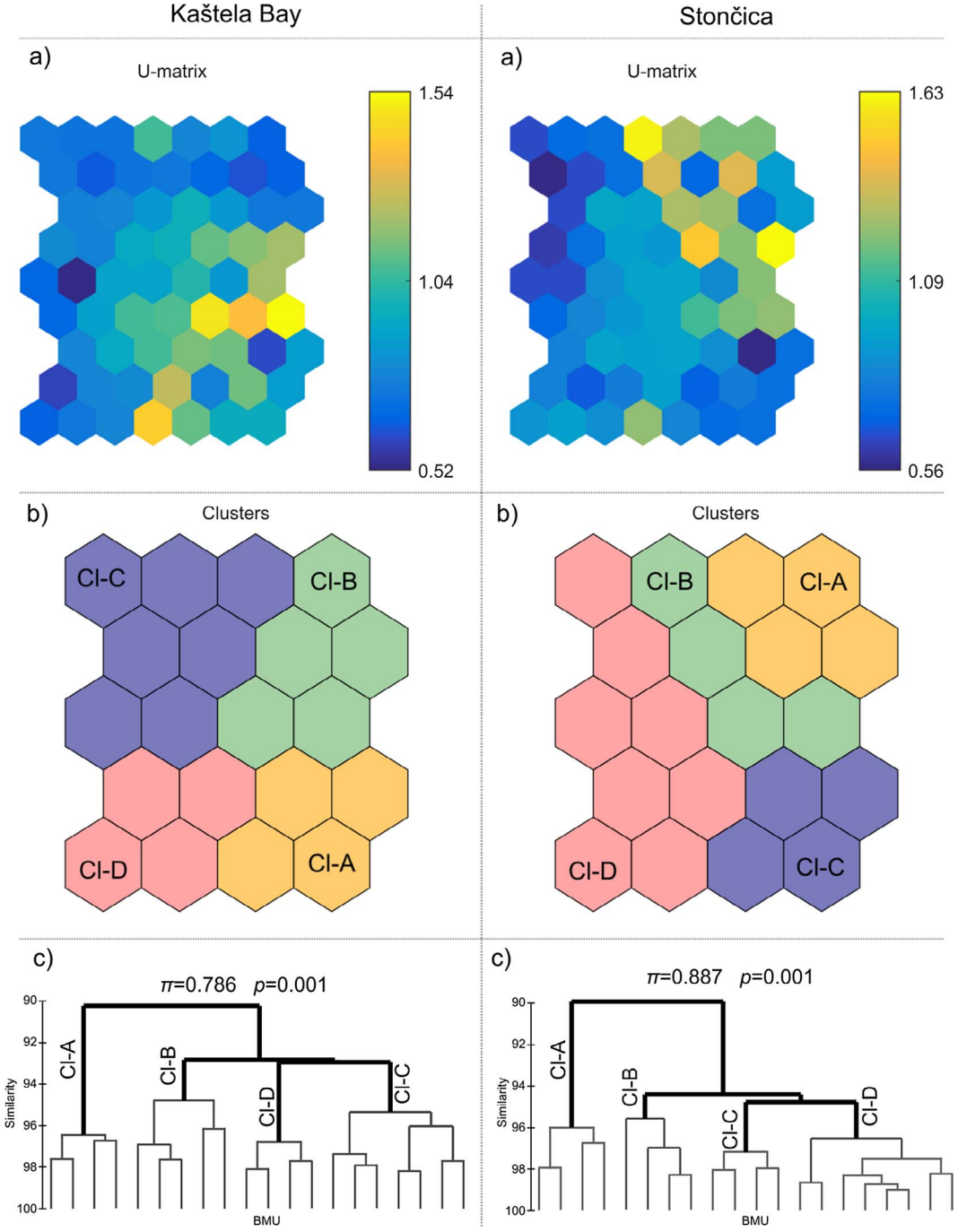

Fig. A1. Clustering through data learning by the self-organizing map. (A) U-matrix, the right band shows the distance between vectors on the SOM plane; (B) Clustering result; (C) Dendrogram of the hierarchical clustering (group average linking) of Best-Matching Units (BMUs) based on group average clustering from Bray-Curtis similarity matrix of standardized and log-transformed biological variables. π statistics resulting from the application of SIMPROOF test showed the only significant separation at p = 0.001 (bold lines) within the investigated BMUs.

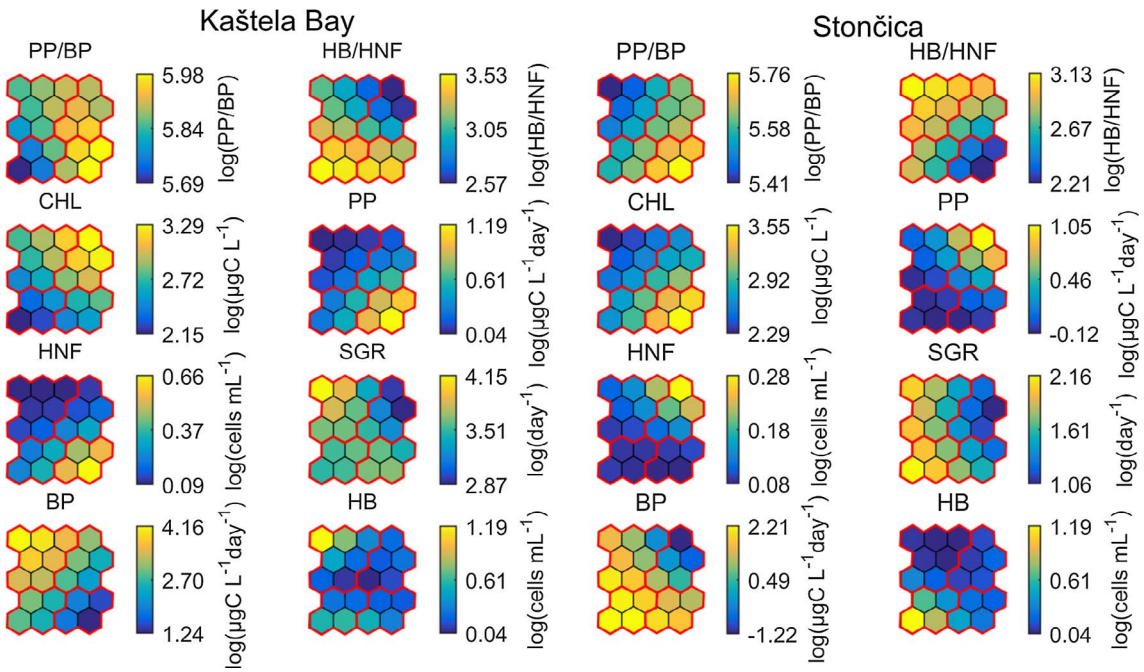

Fig. A2. Component map of all MFW variables used in two self-organizing map (SOM) models, one for the coastal (Kaštela Bay) and second one for the open (Stončica) sea station. Abbrevations: HB – heterotrophic bacteria; BP – bacterial production; SGR – bacterial specific growth rate; HNF – heterotrophic nanoflagellates; PP – primary production; CHL – chlorophyll *a*. The boundaries between clusters are highlighted by red lines.

A.2. SOM models for 2008–2015 period

The results of training both SOM models are shown as U-matrix (Fig. A3a) and trained SOM component map (Fig. A4). The BMU vectors were clustered using Bray-Curtis similarity method (Fig. A3c) into statistically significantly different (p = 0.001) clusters (CL-1, CL-2 and CL-3) and sub-clusters (CL-1A, CL-1B, CL-2A, CL-2B, CL-3A and CL-3B) for both SOM models (Fig. A3b).

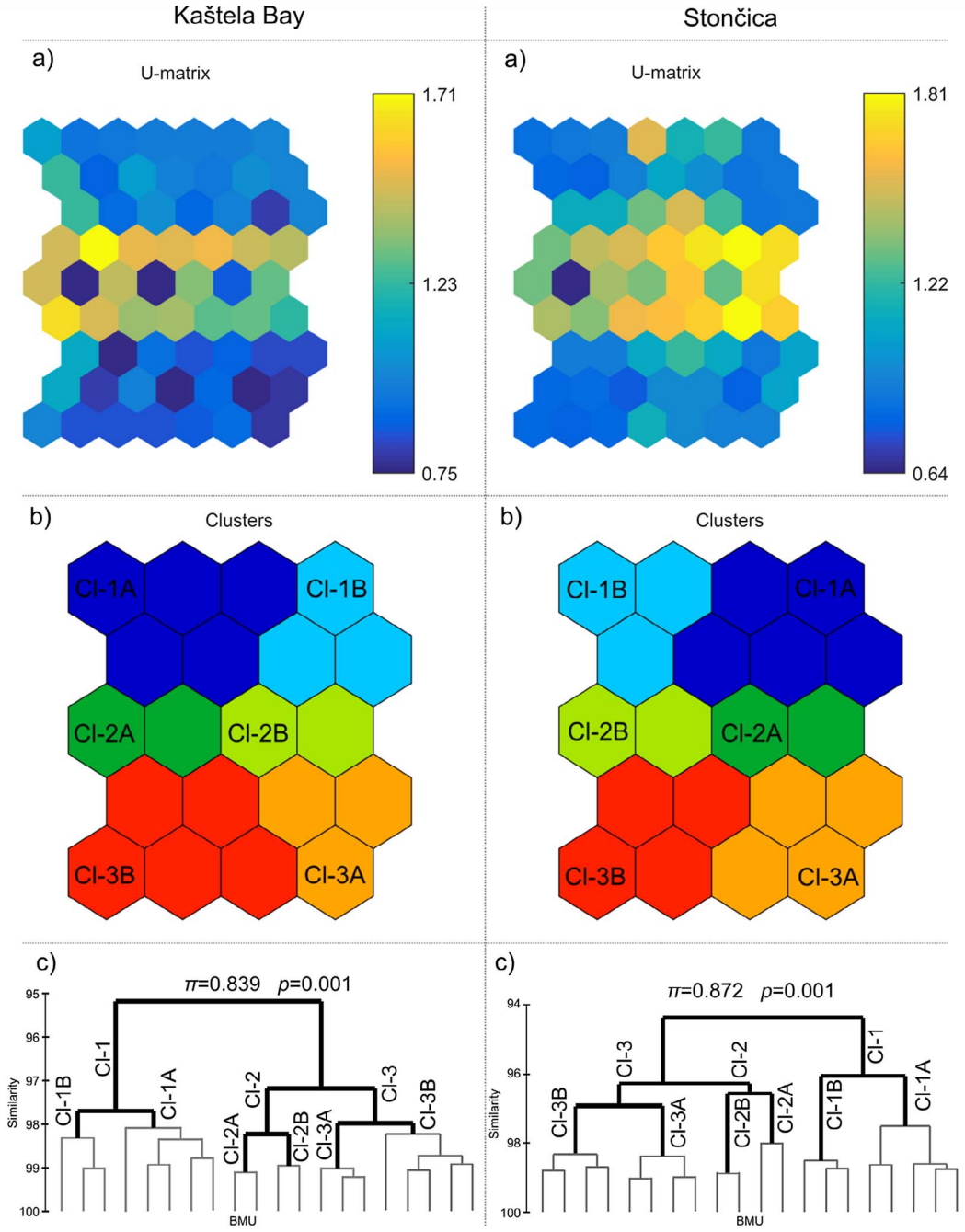

Fig. A3. Clustering through data learning by the self-organizing map. (A) U-matrix, the right band shows the distance between vectors on the SOM plane; (B) Clustering result; (C) Dendrogram of the hierarchical clustering (group average linking) of Best-Matching Units (BMUs) based on group average clustering from Bray-Curtis similarity matrix of standardized and log-transformed biological variables. π statistics resulting from the application of SIMPROOF test showed the only significant separation at p = 0.001 (bold lines) within the investigated BMUs.

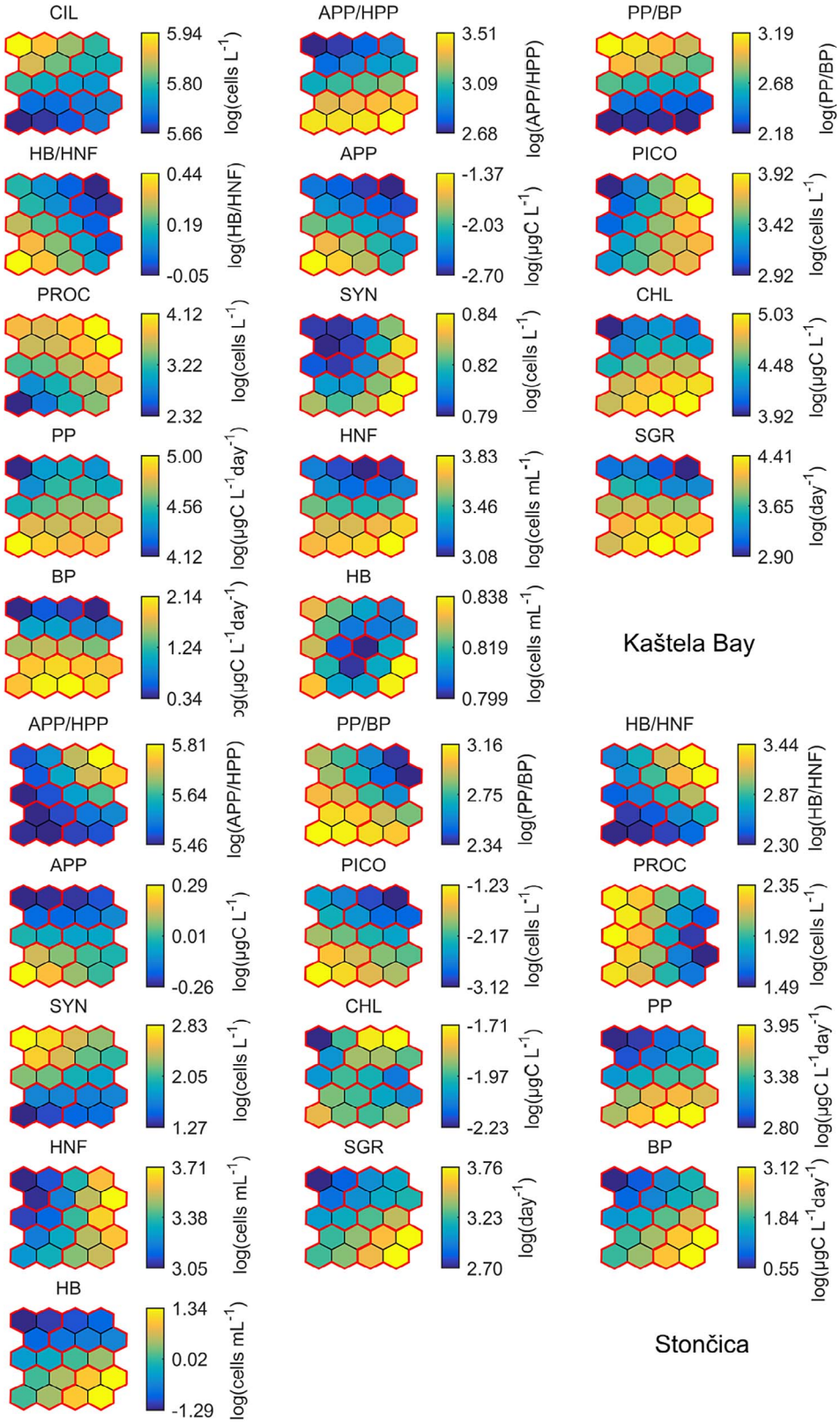

Fig. A4. Component map of all MFW variables used in two self-organizing map (SOM) models, one for the coastal (Kaštela Bay) and second one for the open (Stončica) sea station. Abbrevations: HB – heterotrophic bacteria; BP – bacterial production; SGR – bacterial specific growth rate; HNF – heterotrophic nanoflagellates; PP – primary production; CHL – chlorophyll *a*; SYN – *Synechococcus*; PROC – *Prochlorococcus*; PICO – picoeukaryotes; APP – total autotrophic picoplankton biomass; HPP – heterotrophic picoplankton biomass; CIL – ciliates. The boundaries between clusters are highlighted by red lines.

Appendix B. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.pocean.2018.02.003.

References

Allen, A.P., Gillooly, J.F., Brown, J.H., 2005. Linking the global carbon cycle to individual metabolism. Funct. Ecol. 19, 202–213. http://dx.doi.org/10.1111/j.1365-2435.2005.00952.x.

Apple, J.K., Del Giorgio, P.A., Kemp, W.M., 2006. Temperature regulation of bacterial production, respiration, and growth efficiency in a temperate salt-marsh estuary. Aquat. Microb. Ecol. 43, 243–254.

Bandelj, V., Socal, G., Park, Y.S., Lek, S., Coppola, J., Camatti, E., Capuzzo, E., Milani, L., Solidoro, C., 2008. Analysis of multitrophic plankton assemblages in the Lagoon of Venice. Mar. Ecol. Prog. Ser. 368, 23–40. http://dx.doi.org/10.3354/meps07565.

Beg Paklar, G., Zore-Armanda, M., Dadic, V., 2002. Currents in the Kaštela Bay: empirical analysis and results of a numerical model. Acta Adriat. 43, 33–64.

Behrenfeld, M.J., O'Malley, R.T., Siegel, D.A., McClain, C.R., Sarmiento, J.L., Feldman, G.C., Milligan, A.J., Falkowski, P.G., Letelier, R.M., Boss, E.S., 2006. Climate-driven trends in contemporary ocean productivity. Nature 444, 752–755. http://dx.doi.org/10.1038/nature05317.

Bernacchi, C.J., Singsaas, E.L., Pimentel, C., Portis, A.R., Long, S.P., 2001. Improved temperature response functions for models of Rubisco-limited photosynthesis. Plant, Cell Environ. 24, 253–259. http://dx.doi.org/10.1111/j.1365-3040.2001.00668.x.

Billen, G., Servais, P., Becquevort, S., 1990. Dynamics of bacterioplankton in oligotrophic and eutrophic aquatic environments: bottom-up or top-down control? Hydrobiologia 207, 37–42.

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M., West, G.B., 2004. Toward a metabolic theory of ecology. Ecology 85, 1771–1789. http://dx.doi.org/10.1890/03-9000.

Buitenhuis, E.T., Li, W.K.W., Vaulot, D., Lomas, M.W., Landry, M., Partensky, F., Karl, D.M., Ulloa, O., Campbell, L., Jacquet, S., Lantoine, F., Chavez, F., Macias, D., Gosselin, M., McManus, G.B., 2012. Picophytoplankton biomass distribution in the global ocean. Earth Syst. Sci. Data 4, 37–46. http://dx.doi.org/10.5194/essdd-5-221-2012.

Buljan, M., Zore-Armanda, M., 1979. Hydrographic properties of the Adriatic Sea in the period from 1965–1970. Acta Adriat. 20, 1–368.

Choi, J.Y., Jeong, K.S., Kim, S.K., La, G.H., Chang, K.H., Joo, G.J., 2014. Role of macrophytes as microhabitats for zooplankton community in lentic freshwater ecosystems of South Korea. Ecol. Inf. 24, 177–185. http://dx.doi.org/10.1016/j.ecoinf.2014.09.002.

Choi, J.Y., Kim, S.K., Jeong, K.S., Joo, G.J., 2015. Detecting response patterns of zooplankton to environmental parameters in shallow freshwater wetlands: discovery of the role of macrophytes as microhabitat for epiphytic zooplankton. J. Ecol. Environ. 38, 133–143. http://dx.doi.org/10.5141/ecoenv.2015.015.

Christaki, U., Courties, C., Massana, R., Catala, P., Lebaron, P., Gasol, J.M., Zubkov, M.V., 2011. Optimized routine flow cytometric enumeration of heterotrophic flagellates using SYBR Green I. Limnol. Oceanogr. Methods 9, 329–339. http://dx.doi.org/10.4319/lom.2011.9.329.

Clarke, K.R., Gorley, R.N. (Eds.), 2006. PRIMER v.6 User Manual/Tutorial PRIMER-E, Plymout.

Danovaro, R., Corinaldesi, C., Dell'Anno, A., Fuhrman, J.A., Middelburg, J.J., Noble, R.T., Suttle, C.A., 2011. Marine viruses and global climate change. FEMS Microbiol. Rev. 35, 993–1034. http://dx.doi.org/10.1111/j.1574-6976.2010.00258.x.

Daufresne, M., Lengfellnera, K., Sommer, U., 2009. Global warming benefits the small in aquatic ecosystems. Proc. Natl. Acad. Sci. 106, 12788–12793. http://dx.doi.org/10.1073/pnas.0902080106.

Durrieu de Madron, X., Guieu, C., Sempéré, R., et al., 2011. Marine ecosystems' responses to climatic and anthropogenic forcings in the Mediterranean. Prog. Oceanogr. 91, 97–166. http://dx.doi.org/10.1016/j.pocean.2011.02.003.

Enquist, B.J., Economo, E.P., Huxman, T.E., Allen, A.P., Ignace, D.D., Gillooly, J.F., 2003. Scaling metabolism from organisms to ecosystems. Nature 423, 639–642. http://dx.doi.org/10.1038/nature01671.

Felip, M., Pace, M.L., Cole, J.J., 1996. Regulation of planktonic bacterial growth rates: the effect of temperature and resources. Microb. Ecol. 31, 15–28.

Fonda-Umani, S., Beran, A., 2003. Seasonal variations in the dynamics of microbial plankton communities: first estimates from experiments in the Gulf of Trieste, Northern Adriatic Sea. Mar. Ecol. Prog. Ser. 247, 1–16. http://dx.doi.org/10.3354/meps247001.

Fuhrman, J.A., Azam, F., 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. Mar. Biol. 66, 109–120. http://dx.doi.org/10.1007/BF00397184.

Gačić, M., Dadić, V., Krstulović, N., Marasović, I., Morović, M., Pucher-Petković, T., Sviličić, N., 1987. Near-shore transport processes induced by the wind. Estuar. Coast. Shelf Sci. 24, 35–46. http://dx.doi.org/10.1016/0272-7714(87)90004-7.

Gallina, A.A., Celussi, M., Del Negro, P., 2011. Large-scale distribution and preduction of bacterioplankton in the Adriatic Sea. J. Sea Res. 66, 1–8. http://dx.doi.org/10.4319/lo.2000.45.2.0426.

Giraudel, J.L., Lek, S., 2001. A comparison of self-organizing map algorithm and some conventional statistical methods for ecological community ordination. Ecol. Model. 140, 329–339. http://dx.doi.org/10.1016/S0304-3800(01)00324-6.

Grbec, B., Morović, M., Matić, F., Ninčević Gladan, Ž., Marasović, I., Vidjak, O., Bojanić, N., Čikeš Keč, V., Zorica, B., Kušpilić, G., Matić- Skoko, S., 2015. Climate regime shifts and multi-decadal variability of the Adriatic Sea pelagic ecosystem. Acta Adriat. 56, 47–66.

Gregg, W.W., Conkright, M.E., 2002. Decadal changes in global ocean chlorophyll. Geophys. Res. Lett. 29, 201–204. http://dx.doi.org/10.1029/2002GL014689.

Grob, C., Ulloa, O., Li, W.K.W., Alarcón, G., Fukasawa, M., Watanabe, S., 2007. Picoplankton abundance and biomass across the eastern South Pacific Ocean along latitude 32.5°S. Mar. Ecol. Prog. Ser. 332, 53–62.

Groppe, D.M., Urbach, T.P., Kutas, M., 2011. Mass univariate analysis of event-related brain potentials/fields I: A critical tutorial review. Psychophysiology 48 (12), 1711–1725. http://dx.doi.org/10.1111/j.1469-8986.2011.01273.x.

Hoppe, H.G., Breithaupt, P., Walther, K., Koppe, R., Bleck, S., Sommer, U., Jürgens, K., 2008. Climate warming in winter affects the coupling between phytoplankton and bacteria during the spring bloom: a mesocosm study. Aquat. Microb. Ecol. 51, 105–115. http://dx.doi.org/10.3354/ame01198.

Iriberri, J., Undurrage, A., Muela, A., Egea, L., 1985. Heterotrophic bacterial activity in coastal waters: functional relationship of temperature and phytoplankton population. Ecol. Model. 28, 113–120. http://dx.doi.org/10.1016/0304-3800(85)90016-X.

Kirchman, D.L., Kell, R.G., Simon, M., Welschmeyer, N.A., 1993. Biomass and production of heterotrophic bacterioplankton in the oceanic subarctic Pacific. Deep-Sea Res. I 40, 967–988. http://dx.doi.org/10.1016/0967-0637(93)90084-G.

Kohonen, T., 1982. Self-organized formation of topologically correct feature maps. Biol. Cybern. 69, 59–69. http://dx.doi.org/10.1007/BF00337288.

Kohonen, T., 2001. Self-Organizing Maps, third ed. Springer-Verlag, New York, pp. 501 ISBN: 3540679219.

T. Kohonen, T., 2014. MATLAB Implementations and Applications of the Self-Organizing Map Unigrafia Oy, Helsinki, Finland, 2014. ISBN: 978-952-60-3678-6.

Kovač, Ž., Morović, M., Matić, F., 2014. Uncovering spatial and temporal patterns of Adriatic Sea colour with self-organizing maps. Int. J. Remote Sens. 35, 2105–2117. http://dx.doi.org/10.1080/01431161.2014.885667.

Kriesel, D., 2007. A Brief Introduction to Neural Networks, available at < http://www.dkriesel.com > (accessed 3 February 2016).

Lek, S., Guégan, J.F., 1999. Artificial neural networks as a tool in ecological modelling, an introduction. Ecol. Model. 120, 65–73. http://dx.doi.org/10.1016/S0304-3800(99)00092-7.

Lara, E., Arrieta, J.M., Garcia-Zarandona, I., Boras, J.A., Duarte, C.M., Agustí, S., Wassmann, P.F., Vaqué, D., 2013. Experimental evaluation of the warming effect on viral, bacterial and protistan communities in two contrasting Arctic systems. Aquat. Microb. Ecol. 70, 17–32. http://dx.doi.org/10.3354/ame01636.

Leakey, R.J.G., Burkill, P.H., Sleigh, M.A., 1994. A comparison of fixatives for the estimation of abundance and biovolume of marine planktonic ciliate populations. J. Plankton Res. 16, 375–389. http://dx.doi.org/10.1093/plankt/16.4.375.

Lee, S., Fuhrman, J.A., 1987. Relationships between biovolume and biomass of naturally derived marine bacterioplankton. Appl. Environ. Microbiol. 53, 1298–1303.

Li, W.K.W., Subba Rao, D.V., Harrison, W.G., Smith, J.C., Cullen, J.J., Irwin, B., Platt, T., 1983. Autotrophic picoplankton in the tropical ocean. Science 219, 292–295. http://dx.doi.org/10.1126/science.219.4582.292.

Li, W.K.W., 1998. Annual average abundance of heterotrophic bacteria and *Synechococcus* in surface ocean waters. Limnol. Oceanogr. 43, 1746–1753. http://dx.doi.org/10.4319/lo.1998.43.7.1746.

Li, W.K.W., Harrison, W.G., 2001. Chlorophyll, bacteria and picophytoplankton in ecological provinces of the North Atlantic. Deep-Sea Res. II 48, 2271–2293. http://dx.doi.org/10.1016/S0967-0645(00)00180-6.

Liu, Y., Weisberg, R.H., Mooers, C.N.K., 2006. Performance evaluation of the self-organizing map for feature extraction. J. Geophys. Res. 111, C05018. http://dx.doi.org/10.1029/2005JC003117.

Liu, Y., Weisberg, R.H., 2011. A review of self-organizing map applications in meteorology and oceanography. In: Mwasiagi, J.I. (Ed.), Self-Organizing Maps: Applications and Novel Algorithm Design. InTech, Rijeka, Croatia, pp. 253–272. http://dx.doi.org/10.5772/13146.

López-Urrutia, Â., San Martin, E., Harris, R.P., Irigoien, X., 2006. Scaling the metabolic balance of the oceans. PNAS 103, 8739–8744. http://dx.doi.org/10.1073/pnas.0601137103.

Magazzù, G., Decembrini, F., 1995. Primary production, biomass and abundance of phototrophic picoplankton in the Mediterranean Sea: a review. Aquat. Microb. Ecol. 9, 97–104. http://dx.doi.org/10.3354/ame009097.

Manca, B.B., Kovačević, V., Gačić, M., Viezzoli, D., 2002. Dense water formation in the southern Adriatic Sea in the period 1997–1999. J. Mar. Syst. 33–34, 133–154. http://dx.doi.org/10.1016/S0924-7963(02)00056-8.

Manly, B.F.J., 1997. Randomization, Bootstrap, and Monte Carlo Methods in% Biology, second ed. Chapman and Hall, London.

Maranger, R., Vaqué, D., Nguyen, D., Hébert, M.P., Lara, E., 2015. Pan-Arctic patterns of planktonic heterotrophic microbial abundance and processes: controlling factors and potential impacts of warming. Prog. Oceanogr. 139, 221–232. http://dx.doi.org/10.1016/j.pocean.2015.07.006.

Marie, D., Partensky, F., Jacquet, S., Vaulot, D., 1997. Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. Appl. Environ. Microbiol. 63, 186–193.

Marie, D., Brussaard, C., Partensky, F., Vaulot, D., 1999. Flow cytometric analysis of

phytoplankton, bacteria and viruses. In: Current Protocols in Cytometry. John Wiley & Sons Inc., Ny, pp. 11.11.1–11.11.15.
Matić, F., Grbec, B., Morović, M., 2011. Indications of climate regime shifts in the middle Adriatic Sea. Acta Adriat. 52, 235–246.
Matić, F., Kovač, Ž., Vilibić, I., Mihanović, H., Morović, M., Grbec, B., Leder, N., Džoić, Ž., 2017. Oscillating Adriatic temperature and salinity regimes mapped using the Self-Organizing Maps method. Cont. Shelf Res. 132, 11–18. http://dx.doi.org/10.1016/j.csr.2016.11.006.
Mazzocchi, M.G., Siokou, I., Tirelli, V., Bandelj, V., Fernandez de Puelles, M.L., Ak Őrek, Y., de Olazabal, A., Gubanova, A., Kress, N., Protopapa, M., Solidoro, C., Taglialatela, S., Terbiyik Kurt, T., 2014. Regional and seasonal characteristics of epipelagic mesozooplankton in the Mediterranean Sea based on an artificial neural network analysis. J. Mar. Syst. 135, 64–80.
Meehl, G.A., Stocker, T.F., Collins, W.D., Friedlingstein, P., Gaye, A.T., Gregory, J.M., Kitoh, A., Knutti, R., Murphy, J.M., Noda, A., Raper, S.C.B., Watterson, I.G., Weaver, A.J., Zhao, Z.C., 2007. Global Climate Projections. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L. (Eds.), Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK and New York, NY, USA.
Morán, X.A.G., Calvo-Diaz, A., Ducklow, H.W., 2010. Total and phytoplankton mediated bottom-up control of bacterioplankton change with temperature in NE Atlantic shelf waters. Aquat. Microb. Ecol. 58, 229–239. http://dx.doi.org/10.3354/ame01374.
Ninčević Gladan, Ž., Marasović, I., Grbec, B., Skejić, S., Bužančić, M., Kušpilić, G., Matijević, S., Matic, F., 2009. Inter-decadal variability in phytoplankton community in the Middle Adriatic (Kaštela Bay) in relation to the North Atlantic Oscillation. Estuaries Coasts 33, 376–383. http://dx.doi.org/10.1007/s12237-009-9223-3.
Noble, R.T., Fuhrman, J.A., 1998. Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. Aquat. Microb. Ecol. 14, 113–118.
O'Connor, M.I., Pichler, M.F., Leech, D.M., Anton, A., Bruno, J.F., 2009. Warming and resource availability shift food web structure and metabolism. PLoS Biol. 7, e1000178. http://dx.doi.org/10.1371/journal.pbio.1000178.
Orlić, M., Dadić, V., Grbec, B., Leder, N., Marki, A., Matić, F., Mihanović, H., Beg Paklar, G., Pasarić, M., Pasarić, Z., Vilibić, I., 2007. Wintertime buoyancy forcing, changing seawater properties and two different circulation systems produced in the Adriatic. J. Geophys. Res. – Oceans 112, C03. http://dx.doi.org/10.1029/2005JC003271.
Pandžić, K. (Ed.), 2008. Instructions for observations and measurements at the main meteorological stations. Meteorological and Hydrological Service, Zagreb. 342 pp. (in Croatian).
Park, Y.S., Chon, T.S., Kwak, I.S., Lek, S., 2004. Hierarchical community classification and assessment of aquatic ecosystems using artificial neural networks. Sci. Total Environ. 327, 105–122. http://dx.doi.org/10.1016/j.scitotenv.2004.01.014.
Petchey, O.L., McPhearson, P.T., Casey, T.M., Morin, P.J., 1999. Environmental warming alters food-web structure and ecosystem function. Nature 402, 69–72. http://dx.doi.org/10.1038/47023.
Pomeroy, L.R., Wiebe, W.J., 2001. Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria. Aquat. Microb. Ecol. 23, 187–204.
Proctor, I.M., Okubo, A., Fuhrman, J.A., 1993. Calibrating estimates of phage-induced mortality in marine bacteria: ultrastructural studies of marine bacteriophage development from one-step growth experiments. Microb. Ecol. 25, 161–182. http://dx.doi.org/10.1007/BF00177193.
Ratkowsky, D.A., Olley, J., McMeekin, T.A., Ball, A., 1982. Relationship between temperature and growth rate of bacterial cultures. J. Bacteriol. 149, 1–5.
Rivkin, R.B., Anderson, M.R., Lajzerowicz, C., 1996. Microbial processes in cold oceans. I. Relationship between temperature and bacterial growth rate. Aquat. Microb. Ecol. 10, 243–254. http://dx.doi.org/10.3354/ame010243.
Rivkin, R.B., Legendre, L., 2001. Biogenic carbon cycling in the upper ocean: effects of microbial respiration. Science 291, 2398–3240. http://dx.doi.org/10.1126/science.291.5512.2398.
Sarmiento, J.L., Hughes, T.M.C., Stouffer, R.J., Manabe, S., 1998. Simulated response of the ocean carbon cycle to anthropogenic climate warming. Nature 393, 245–249. http://dx.doi.org/10.1038/30455.
Sarmento, H., Montoya, J.M., Vázquez-Domínguez, E., Vaqué, D., Gasol, J.M., 2010. Warming effects on marine microbial food web processes: how far can we go when it comes to predictions? Philosoph. Transact. Roy. Soc. Lond. B: Biol. Sci. 365, 2137. http://dx.doi.org/10.1098/rstb.2010.0045.
Schroeder, K., Chiggiato, J., Bryden, H.L., Ben Ismail, S., 2016. Abrupt climate shift in the Western Mediterranean Sea. Sci. Rep. 6, 23009. http://dx.doi.org/10.1038/srep23009.
Shaltout, M., Omstedt, A., 2014. Recent sea surface temperature trends and future scenarios for the Mediterranean Sea. Oceanologia 56, 411–443. http://dx.doi.org/10.5697/oc.56-3.411.
Shiah, F., Ducklow, H.W., 1994. Temperature regulation of heterotrophic bacterioplankton abundance, production, and specific growth rate in Chesapeake Bay. Limnol. Oceanogr. 39, 1243–1258. http://dx.doi.org/10.4319/lo.1994.39.6.1243.
Skliris, N., Sofianos, S., Gkanasos, A., Mantziafou, A., Vervatis, V., Axaopoulos, P., Lascaratos, A., 2011. Decadal scale variability of sea surface temperature in the Mediterranean Sea in relation to atmospheric variability. Ocean Dyn. 62, 13–30. http://dx.doi.org/10.1007/s10236-011-0493-5.
Socal, G., Acri, F., Bastianini, M., Bernardi Aubry, F., Bianchi, F., Cassin, D., Coppola, J., De Lazzari, A., Bandelj, V., Cossarini, G., Solidoro, C., 2008. Mar. Ecol. 29, 449–468. http://dx.doi.org/10.1111/j.1439-0485.2008.00266.x.
Solidoro, C., Bandelj, V., Barbieri, P., Cossarini, G., Fonda Umani, S., 2007. Understanding dynamic of biogeochemical properties in the northern Adriatic Sea by using self-organizing maps and k-means clustering. J. Geophys. Res. 112, C07S90.
Solidoro, C., Bastianini, M., Bandelj, V., Codermatz, R., Cossarini, G., Melaku Canu, D., Ravagnan, E., Salon, S., Trevisani, S., 2009. Current state, scales of variability, and trends of biogeochemical properties in the northern Adriatic Sea. J. Geophys. Res. 114, C07S91.
Steemann-Nielsen, E., 1952. The use of radioactive carbon (^{14}C) for measuring organic production in the sea. Journal du Conseil / Conseil Permanent International pour l'Exploration de la Mer 18, 117–140. http://dx.doi.org/10.1093/icesjms/18.2.117.
Strecker, A.L., Cobb, T.P., Vinebrooke, R.D., 2004. Effects of experimental greenhouse warming pn phytoplankton and zooplankton communities in fishless alpine ponds. Limnol. Oceanogr. 49, 1182–1190. http://dx.doi.org/10.4319/lo.2004.49.4.1182.
Strickland, J.D.H., Parsons, T.R., 1972. A practical handbook of seawater analysis. Bull. Fisher. Res. Board Canada 167, 1–311. http://dx.doi.org/10.1002/iroh.19700550118.
Supić, N., Orlić, M., 1992. Annual cycles of sea surface temperature along the east Adriatic coast. Geofizika 9, 79–97.
Šantić, D., Krstulović, N., Šolić, M., Ordulj, M., Kušpilić, G., 2013. Dynamics of prokaryotic picoplankton community in the central and southern Adriatic Sea (Croatia). Helgol. Mar. Res. 67, 471–481. http://dx.doi.org/10.1007/s10152-012-0336-x.
Šolić, M., Krstulović, N., 1994. The role of predation in controlling bacterial and heterotrophic nanoflagellate standing stocks in the coastal Adriatic Sea: seasonal patterns. Mar. Ecol. Prog. Ser. 114, 219–235. http://dx.doi.org/10.3354/meps114219.
Šolić, M., Krstulović, N., 1995. Bacterial carbon flux through microbial loop in Kaštela Bay (Adriatic Sea). Ophelia 41, 345–360. http://dx.doi.org/10.1080/00785236.1995.10422052.
Šolić, M., Krstulović, N., Vilibić, I., Kušpilić, G., Šestanović, S., Šantić, D., Ordulj, M., 2008. The role of water mass dynamics in controlling bacterial abundance and production in the middle Adriatic Sea. Mar. Environ. Res. 65, 388–404.
Šolić, M., Krstulović, N., Vilibić, I., Bojanić, N., Kušpilić, G., Šestanovic, S., Šantic, D., Ordulj, M., 2009. Variability in the bottom-up and top-down control of bacteria on trophic and temporal scale in the middle Adriatic Sea. Aquat. Microb. Ecol. 58, 15–29 doi: 0.3354/ame01342.
Šolić, M., Krstulović, N., Kušpilić, G., Ninčević Gladan, Ž., Bojanić, N., Šestanović, S., Šantić, D., Ordulj, N., 2010. Changes in microbial food web structure in response to changed environmental trophic status: a case study of the Vranjic Basin (Adriatic Sea). Mar. Environ. Res. 70, 239–249. http://dx.doi.org/10.1016/j.marenvres.2010.05.007.
Šolić, M., Krstulović, N., Šantić, D., Šestanović, S., Ordulj, M., Bojanić, N., Kušpilić, G., 2015. Structure of microbial communities in phosphorus-limited estuaries along the eastern Adriatic coast. J. Mar. Biol. Assoc. United Kingdom 95, 1565–1578. http://dx.doi.org/10.1017/S0025315415000442.
Šolić, M., Krstulović, N., Šantić, D., Šestanović, S., Kušpilić, G., Bojanić, N., Ordulj, M., Jozić, S., Vrdoljak, A., 2017. Impact of 3°C temperature rise on bacterial growth and carbon transfer towards higher trophic levels: empirical models for the Adriatic Sea. J. Mar. Syst. http://dx.doi.org/10.1016/j.jmarsys.2017.01.001.
Tsai, A.Y., Gong, G.C., Shiau, W., 2015. Viral lysis and nanoflagellate grazing on prokaryotes: effects of short-term warming in a coastal subtropical marine system. Hydrobiologia 751, 43–54. http://dx.doi.org/10.1007/s10750-014-2170-4.
Utermöhl, H., 1958. Zur Vervollkommnung der quantitativen Phytoplankton – Methodik. Mitteilungen Internationale Vereinigung für Theoretische und Angewandte Limnologie 9, 1–37.
Vaqué, D., Gasol, J.M., Marrasé, C., 1994. Grazing rates on bacteria: the significance of methodology and ecological factors. Mar. Ecol. Prog. Ser. 109, 263–274. http://dx.doi.org/10.3354/meps109263.
Vázquez-Domínguez, E., Vaqué, D., Gasol, J.M., 2012. Temperature effects on the heterotrophic bacteria, heterotrophic nanoflagellates, and microbial top predators of the NW Mediterranean. Aguatic Microb. Ecol. 67, 107–121 doi: https//doi.org710.3354/ame01583.
Vesanto, J., Alhoniemi, E., 2000. Clustering of the self-organizing map. IEEE Trans. Neural Networks 11, 586–600. http://dx.doi.org/10.1109/72.846731.
West, G.B., Brown, J.H., Enquist, B.J., 1997. A general model for the origin of allometric scaling laws in biology. Science 276, 122–126. http://dx.doi.org/10.1126/science.276.5309.122.
White, P.A., Kalff, J., Rasmussen, J.B., Gasol, J.M., 1991. The effect of temperature and algal biomass on bacterial production and specific growth rate in freshwater and marine habitats. Microb. Ecol. 21, 99–118. http://dx.doi.org/10.1007/BF02539147.
Wojtal-Frankiewicz, A., Kruk, A., Frankiewicz, P., Oleksińska, Z., Izydorczyk, K., 2015. Long-term patterns in the population dynamics of *Daphnia longispina*, *Leptodora kindtii* and cyanobacteria in a shallow reservoir: a self-organising map (SOM) approach. PloS ONE 10 (12), e0144109. http://dx.doi.org/10.1371/journal. pone.0144109.
Yvon-Durocher, G., Jones, J.I., Trimmer, M., Woodward, C., Montoya, J.M., 2010. Warming alters the metabolic balance of ecosystems. Philosoph. Transact. Roy. Soc. B 365, 2117–2126. http://dx.doi.org/10.1098/rstb.2010.
Zore-Armanda, M., 1980. Some dynamic and hydrographic properties of the Kaštela Bay. Acta Adriat. 21, 55–74.